REMARKS

Claims 40-68 were pending prior to this response. By the present communication, claims 42-44, 47-53, 56, 57, 64 and 65 are cancelled without prejudice. In addition, to define Applicants' invention with greater particularity, claims 40, 45, 54, and 62 have been amended and new claims 69-83 have been added. The new claim language adds no new matter, being fully disclosed in the Specification and original claims. Accordingly claims 40, 41, 45, 46, 54, 55, 58-63, and 66-83 are currently pending.

The Drawings

The Office Action indicates that submission of new formal drawings is required for this response to be considered responsive under MPEP, Section 608.02(c). Accordingly, Applicants submit herewith as Exhibit B a set of new formal drawings for this application, thereby fulfilling the provisions of MPEP, Section 608.02(c).

The Claim Objection

The Examiner objects to claim 51 as allegedly containing an informality in the lack of the article "the" before "nucleic acid" in line 2. To overcome the objection, claim 51 has been amended to insert "the" prior to "nucleic acid" in claim 51. Accordingly, reconsideration and withdrawal of the objection to claim 51 are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants traverse the rejection of claims 47-68 under 35 U.S.C. 112, Second Paragraph, for allegedly being indefinite. With regard to claim 47, line 1, the Examiner alleges that the phrase "treating a condition associated with autoimmune diabetes" lacks description in the Specification. By the present communication, claims 47-52 have been cancelled without prejudice, thus rendering the rejection moot as to these claims. In addition, claims 54 and 62 have been amended to delete the phrase "condition associated with". Claim 54 now recites "treating autoimmune diabetes" and claim 62 has been amended to recite "treating an autoimmune process associated with the autoimmune diabetes".

With regard to claims 47, 54 and 62, the Examiner asserts that improper Markush format is used, allegedly rending the claim scope or clarity ambiguous. Claim 47 has been cancelled, rendering the rejection moot as to claim 47. In addition, claims 54 and 62 have been amended to remove alternative expressions, making the issue of proper alternative claiming format irrelevant to claims 47, 54 and 62.

In view of these amendments, Applicants respectfully submit that claims 47-68 meet all requirements under 35 U.S.C. § 112, Second Paragraph, and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 102

Applicants respectfully traverse the rejection of claims 40, 41, 43-45, 47, 50, 51, 53, 54, 57, 58, 60, 62, 65 and 66 under 35 U.S.C. § 102 (b) as allegedly being anticipated by Tokui et al. (Bunshi Tonyobyogaku Vo, hereinafter "Tokui"). Claims 47-51 have been cancelled by amendment herein, making the rejection moot as to the subject matter of these claims. In addition, Applicants respectfully submit that the invention methods and compositions for treating autoimmune diabetes, as recited by amended claims 40, 54 and 62, distinguish over the disclosure of Tokui by requiring one or more polynucleotide constructs encoding the combination of GAD self-antigen and IL-10. In the method of treatment claims, the polynucleotide constructs are required to be administered to the subject as naked DNA or in a colloidal dispersion system. Tokui fails to disclose administration to a subject of polynucleotides encoding the combination of GAD self-antigen and IL-10, by any method of administration. Therefore, Applicants respectfully submit that Tokui fails to disclose each and every element of claims 40, 41, 43-45, 54, 57, 58, 60, 62, 65 and 66, as would be required to support a rejection under 35 U.S.C. § 102(b). Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 40-68 under 35 U.S.C. § 103 as allegedly being unpatentable over Tokui in view of U.S. Patent 5,891,435 to Muir (hereinafter "Muir"). Claims 47-51 are now cancelled. Applicants submit that the invention compositions and methods, as defined by the claims as amended herein, distinguish over the combined disclosures of Tokui and Muir by requiring use of polynucleotides encoding the combination of GAD self-antigen and IL-10. Similarly the new claims presented herein, claims 69-83,

distinguish over the combined disclosures of Tokui and Muir by requiring use of polynucleotides encoding either the combination of insulin B chain antigen and IL-10, or the combination of insulin B chain self-antigen and IL-4 in treatment of autoimmune diabetes. Use of a viral vector is not required.

A. Applicants disagree with the Examiner's assertion that Muir's disclosure regarding the role of interleukins in suppression of autoimmune diabetes would have motivated those of skill in the art to combine the teaching of Tokui and Muir to arrive at Applicants' claimed invention (Office Action, page 6). Tokui's disclosure pertains to administration of the combination of GAD and IL-4 via viral vector. Muir's disclosure pertains to use of the combination of insulin B chain and an adjuvant, such as incomplete Freund's adjuvant. Use of viral vectors for administration of DNA is disclosed also by Muir. However Muir is absolutely silent regarding administration of a polynucleotide encoding IL-10 or IL-4 in combination with one encoding any self-antigen.

Muir's comments regarding IL-10 are in the context of a more general statement regarding a number of different immunosuppressive actors, including "(1) transforming growth factor-β (TGF-β), (2) competition for antigen binding between non-destructive and destructive cells recognizing the same antigen, (3) stimulation of 'anti-idiotypic' T lymphocytes that bind the TCR of destructive T lymphocytes, and (4) activation of 'suppressor-inducer' lymphocytes." The Examiner has provided no reasons to support the assertion that those of skill in the art would extract from Muir the suggestion to replace IL-4 with IL-10 in a GAD-based DNA vaccine or to combine polynucleotides encoding insulin B chain -antigen with those encoding an interleukin,

such as IL-10, or IL-4 rather than, for example, a polynucleotide encoding TGF-β as disclosed by Muir.

Moreover, Applicants disagree with the Examiner's assertion that IL-4 and IL-10 are listed as equivalents in the Specification (Office Action, page 6). To the contrary, Applicants disclose:

> ... it would be desirable to selectively stimulate the production of immunomodulator compounds such as, for example, cytokines like IL-4, IL-10, IL-9, IL-13 and TGF-B. It will be appreciated that the induction of such immunomodulator compounds may be associated with the identity of the selected epitope in the context of the T cell repertoire, the cytokine context during priming and the inoculation regimen/antigen timing and duration of inoculations.

(Specification, page 6, lines 9-15). Thus, far from suggesting the IL-4 and IL-10 are interchangeable in function, Applicants underline that the choice is context specific. Moreover, those of skill in the art were aware at the filing of the present application that IL-4 and IL-10 display different immunomodulatory effects. Applicants submit for consideration by the Examiner a print out containing abstracts of articles published prior to the filing of the present application, each of which discusses differences between IL-10 and IL-4. In view of the teachings of the art and in the Specification, Applicants respectfully submit that those of skill in the art would not consider that IL-4 and IL-10 are functional equivalents.

Thus, Applicants respectfully submit that the Examiner has failed to establish prima facie obvious of claims 52-68 or new claims 69-83 under 35 U.S.C. § 103 over the combined disclosures of Tokui and Muir. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

In view of the above amendments and remarks, Applicants submit that all objection and rejections have now been overcome and allowance of claims 40, 41, 45, 46, 54, 55, 58-63, and 66-83 is respectfully requested. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: October 1, 2002

Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

USPTO CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosure: Exhibit A
Exhibit B – Formal drawings
Print out of abstracts re IL-10 and IL-4

Applicants: Matthias G. von Herrath
Application No.: 09/336,672
Filed: June 17, 1999
Exhibit A: Page 1

EXHIBIT A

Version with Markings to Show Changes Made

In the Claims

Please cancel claims 42-44, 47-53, 56, 57, 64 and 65 without prejudice .

Please amend claims 40, 45, 54 and 62 to read as follows:

40. (Amended) An immunomodulating composition for treating a condition or autoimmune process associated with autoimmune diabetes, said composition comprising one or more nucleic acid construct encoding [a] GAD self-antigen [selected from GAD, insulin B-chain, and a combination thereof and a cytokine selected from] and IL-10 [, IL4, and a combination thereof,] in a pharmaceutically acceptable carrier.

45. (Amended) The composition of claim [40] 44, wherein the nucleic acid construct further comprises a regulatory element operatively linked to nucleic acid encoding the self-antigen or the [cytokine] IL-10.

54. (Amended) A method for treating autoimmune diabetes in a subject in need thereof comprising administering to the subject by peripheral administration an immunomodulatory effective amount of one or more [plasmids expressing a] nucleic acid construct encoding [a] GAD self-antigen [selected from insulin B chain, GAD, and a combination thereof and a cytokine selected from IL-4,] and IL-10 [, and a combination thereof,] in a pharmaceutically acceptable carrier, wherein transient expression of the self-antigen and the [cytokine] IL-10 in the subject treats the [condition associated with the] autoimmune diabetes.

Applicants: Matthias G. von Herrath
Application No.: 09/336,672
Filed: June 17, 1999
Exhibit A: Page 2

62. (Amended) A method for treating an autoimmune process associated with autoimmune diabetes in a subject in need thereof comprising administering to the subject by peripheral administration an immunomodulatory effective amount of one or more [plasmids expressing a] nucleic acid construct encoding [a] GAD self-antigen [selected from insulin B chain, GAD, and a combination thereof] and [a cytokine selected from IL-4,] IL-10 [, and a combination thereof,] in a pharmaceutically acceptable carrier, wherein transient expression of the self-antigen and the [cytokine] IL-10 in the subject treats the autoimmune process associated with the autoimmune diabetes.

Please add the following new claims 69-83:

-- 69. (New) An immunomodulating composition for treating a condition or autoimmune process associated with autoimmune diabetes, said composition comprising one or more nucleic acid construct encoding an insulin B-chain self-antigen and a cytokine selected from the group consisting of IL-10, IL-4, and a combination thereof, in a pharmaceutically acceptable carrier.

70. (New) The composition of claim 69, wherein the autoimmune diabetes is type I diabetes.

71. (New) The composition of claim 71, wherein the nucleic acid construct further comprises a regulatory element operatively linked to the nucleic acid encoding the self-antigen or the cytokine.

72. (New) The composition of claim 72, wherein the regulatory element is a promoter selected from the group consisting of Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus Long Terminal Repeat (HIV LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) promoter, human Actin, human Myosin, RSV, human Hemoglobin, human muscle creatine and EBV.

73. (New) A method for treating autoimmune diabetes in a subject in need thereof comprising administering to the subject by peripheral administration an immunomodulatory effective amount of one or more nucleic acid construct encoding insulin B chain self-antigen and a cytokine selected from the group consisting of IL-4, IL-10, and a combination thereof, in a pharmaceutically acceptable carrier, wherein transient expression of the self-antigen and the cytokine in the subject treats the autoimmune diabetes.

74. (New) The method of claim 76, wherein the subject is a human.

75. (New) The method of claim 76, wherein the nucleic acid construct further comprises a regulatory element operatively to the nucleic acid encoding the self-antigen or the cytokine.

76. (New) The method of claim 78, wherein the regulatory element is a promoter selected from the group consisting of Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus Long Terminal Repeat (HIV LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) promoter, human Actin, human Myosin, RSV, human Hemoglobin, human muscle creatine and EBV.

77. (New) The method of claim 71, wherein the treatment comprises controlling the blood sugar of the subject.

78. (New) A method for treating an autoimmune process associated with autoimmune diabetes in a subject in need thereof comprising administering to the subject by peripheral administration an immunomodulatory effective amount of one or more nucleic acid construct encoding insulin B-chain self-antigen and a cytokine selected from the group consisting of IL-4, IL-10 , and a combination thereof, in a pharmaceutically acceptable carrier, wherein transient expression of the self-antigen and the cytokine in the subject treats the autoimmune process associated with the autoimmune diabetes.

79. (New) The method of claim 78, wherein the subject is a human.

80. (New) The method of claim 78, wherein the nucleic acid construct further comprises a regulatory element operatively linked to the nucleic acid encoding the self-antigen or the cytokine.

81. (New) The method of claim 80, wherein the regulatory element is a promoter selected from the group consisting of Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus Long Terminal Repeat (HIV LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) promoter, human Actin, human Myosin, RSV, human Hemoglobin, human muscle creatine and EBV.

82. (New) The method of claim 80, wherein the treatment comprises induction of T-cells reactive to the self-antigen.

83. (New) The method of claim 80, wherein the nucleic acid construct is naked DNA. --

Int Immunol 1992 May;4(5):563-9

Differential effects of IL-4 and IL-10 on IL-2-induced IFN-gamma synthesis and lymphokine-activated killer activity.

Hsu DH, Moore KW, Spits H.

Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304.

Culture of human peripheral blood mononuclear cells (PBMC) with IL-2 stimulates synthesis of cytokines and generation of lymphokine-activated killer (LAK) activity. Both IL-4 and IL-10 [cytokine synthesis inhibitory factor (CSIF)] inhibit IL-2-induced synthesis of IFN-gamma and tumor necrosis factor (TNF)-alpha by human PBMC. However, unlike IL-4, IL-10 inhibits neither IL-2-induced proliferation of PBMC and fresh natural killer (NK) cells, nor IL-2-induced LAK activity. Moreover, IL-4 inhibits IL-2-induced IFN-gamma synthesis by purified fresh NK cells, while in contrast the inhibitory effect of IL-10 is mediated by CD14+ cells (monocytes/macrophages). IL-10 inhibits TNF-alpha synthesis by monocytes or monocytes plus NK cells, but not by NK cells alone. These results suggest that IL-4 and IL-10 act on NK cells via distinct pathways, and that IL-2-induced cytokine synthesis and LAK activity are regulated via different mechanisms.

J Leukoc Biol 1995 Feb;57(2):303-9

Differential effects of anti-inflammatory cytokines (IL-4, IL-10 and IL-13) on tumoricidal and chemotactic properties of human monocytes induced by monocyte chemotactic and activating factor.

Yano S, Sone S, Nishioka Y, Mukaida N, Matsushima K, Ogura T.

Third Department of Internal Medicine, University of Tokushima School of Medicine, Japan.

The effect of recombinant human IL-4, IL-10, and IL-13 on the chemotaxis and antitumor activity of human blood monocytes induced by monocyte chemotactic and activating factor (MCAF) was examined. MCAF alone did not induce monocyte-mediated cytotoxicity against human melanoma (A375-M) cells whereas it significantly enhanced the cytotoxicity by norMDP-stimulated monocytes. MCAF, unlike IFN-gamma, had no priming effect on monocyte activation by norMDP. MCAF acted with norMDP or LPS to enhance the production of both IL-1 beta and TNF-alpha. Enhanced cytotoxicity of monocytes stimulated with MCAF plus norMDP was reduced by IL-1 receptor antagonist and anti-TNF-alpha antibody. IL-4, IL-10, and IL-13 suppressed the generation of

antitumor activity and cytokine production (IL-1 beta and TNF-alpha) of monocytes stimulated with MCAF plus norMDP or LPS. Chemotaxis of monocytes induced by MCAF was not affected by norMDP or any of the anti-inflammatory cytokines (IL-4, IL-10, and IL-13). Moreover, the pretreatment of monocytes with anti-inflammatory cytokines did not suppress monocyte-chemotaxis. These findings suggest that in vivo recruitment and anti-tumor expression of blood monocytes induced by MCAF may be differently regulated by anti-inflammatory cytokines in vivo.

J Leukoc Biol 1995 Mar;57(3):450-4

Differential inhibitory effects of interleukin-10, interleukin-4, and dexamethasone on staphylococcal enterotoxin-induced cytokine production and T cell activation.

Krakauer T.

Applied Research Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011.

The cytokine profile of human peripheral blood mononuclear cells (PBMC) stimulated by staphylococcal enterotoxin (SE) A and B was examined. Production of tumor necrosis factor (TNF alpha), interleukin (IL)-1, IL-6, IL-2, and gamma interferon (IFN-gamma) was observed. In contrast, Th2 cytokines IL-4 and IL-10 were absent from SEA- or SEB-stimulated PBMC. Moreover, adding IL-10 to SE-stimulated PBMC inhibited the production of IL-1, IL-6, TNF alpha, and IFN gamma by 50 to 80% but had less effect (8-30%) on T cell proliferation. IL-4 was less effective than IL-10 in inhibiting cytokine production and enhanced T cell proliferation by SEA or SEB. The anti-inflammatory agent, dexamethasone, was the most potent agent in controlling the SE-mediated effects as evidenced by inhibited T cell proliferation (55%) and reduced levels of IL-1, IL-6, and IFN gamma (60% to 100%) and TNF alpha (50%). Reducing levels of toxic mediators such as TNF alpha, IL-1, IL-6, and IFN gamma by dexamethasone in SE-induced T cell responses may be a useful therapeutic strategy to circumvent SE toxicity and pathogenesis.

J Immunol 1996 Apr 1;156(7):2591-8

Differential regulation of IL-6 gene transcription and expression by IL-4 and IL-10 in human monocytic cell lines.

Takeshita S, Gage JR, Kishimoto T, Vredevoe DL, Martinez-Maza O.

UCLA School of Nursing, UCLA School of Medicine, Los Angelas, 90095, USA.

IL-4 and IL-10 inhibit the cytokine production and mRNA expression by monocytes/macrophages. To investigate the molecular mechanism of the inhibitory effect on transcriptional or post-transcriptional regulation of IL-6 gene expression by IL-4 and IL-10, we studied IL-6 production, expression level of IL-6 mRNA, IL-6 promoter activity, transcriptional activity of NF-kappaB and NF-IL-6, and IL-6 mRNA stability in human monocytic cell lines, THP-1 and U937, stimulated by PMA and LPS in the absence or the presence of IL-4 or IL-10. Both IL-4 and IL-10 were seen to inhibit IL-6 production and the expression of IL-6 mRNA in both monocytic cell lines studied. In chloramphenicol acetyltransferase assays, utilizing the transient transfection of a chloramphenicol acetyltransferase reporter plasmid containing the IL-6 gene promoter, IL-4, but not IL-10, suppressed the transcriptional activity of the IL-6 gene promoter stimulated by PMA and LPS. Electrophoretic mobility shift assays showed that IL-4, but not IL-10, inhibited nuclear NF-kappaB activity, and that IL-4 and IL-10 did not affect NF-IL-6 activity. On the other hand, IL-10 enhanced the degradation of IL-6 mRNA in a mRNA stability assay. These results suggest that IL-4 may inhibit the transcription of the IL-6 gene by affecting NF-kappaB binding activity, while IL-10 may inhibit the IL-6 mRNA levels post-transcriptionally, without suppressing promoter activity. Therefore, we conclude that IL-4 and IL-10 inhibit IL-6 production by different mechanisms in human monocytic cell lines.

Biochim Biophys Acta 1999 Feb 4;1449(1):83-92

Interleukin-4 (IL-4), but not IL-10, regulates the synthesis of IL-6, IL-8 and leukemia inhibitory factor by human bone marrow stromal cells.

Denizot Y, Besse A, Raher S, Nachat R, Trimoreau F, Praloran V, Godard A.

Laboratoire d'Hematologie Experimentale, Faculte de Medecine, 2 rue Dr. Marcland, 87025, Limoges, France.

Leukemia inhibitory factor (LIF), interleukin 6 (IL-6) and IL-8 are important regulators of inflammation and hematopoiesis. Human bone marrow stromal cells regulate marrow hematopoiesis by secreting cytokines. By using reverse-transcriptase polymerase chain reaction (RT-PCR), we demonstrate that human bone marrow stromal cells constitutively express LIF, IL-6 and IL-8 transcripts. By using specific ELISAs, we found that their spontaneous productions of LIF, IL-6 and IL-8 are elevated in response to serum and after stimulation with the pro-inflammatory cytokines IL-1alpha and TNF-alpha. The anti-inflammatory cytokine IL-4 reduces their serum- and cytokine-induced LIF secretion. By contrast, IL-4 stimulates their serum- and IL-1alpha-induced IL-6 synthesis. IL-4 has no effect on the serum-induced IL-8 synthesis by marrow stromal cells, but stimulates their cytokine-induced IL-8 production. The anti-inflammatory cytokine IL-10 has no effect on the serum- and cytokine-induced LIF, IL-6 and

IL-8 synthesis by bone marrow stromal cells. RT-PCR experiments reveal the presence of IL-4 receptor alpha-chain mRNA and IL-10 receptor mRNA in cultured bone marrow stromal cells. The differential regulation by IL-4 of two related cytokines, such as LIF and IL-6, and the enhanced effect of this 'anti-inflammatory' cytokine on IL-6 and IL-8 synthesis highlight the tightly controlled regulation and the complexity of the cytokine production within the human bone marrow.

Int Immunol 1998 Jun;10(6):757-65 Related Articles, Links

Differential chemokine response of murine macrophages stimulated with cytokines and infected with Listeria monocytogenes.

Flesch IE, Barsig J, Kaufmann SH.

Department of Immunology, University of Ulm, Germany.

During inflammatory processes the infected macrophage is a rich source of chemokines which induce infiltration of leukocytes to the site of infection. We investigated the regulation of chemokine production by murine macrophages in response to infection with the intracellular bacterial pathogen, Listeria monocytogenes. As a source of quiescent macrophages, murine bone marrow-derived macrophages (BMM) cultured under serum-free conditions were used. With RT-PCR, we detected induction of RNA message for the chemokines macrophage inflammatory protein (MIP)-2, KC, MIP-1alpha, MIP-1beta, IFN-gamma-inducible protein-10 and RANTES in L. monocytogenes-infected macrophages. Accordingly, ELISA-detectable MIP-1alpha, MIP-2 and KC protein was induced by infection with L. monocytogenes. In contrast, L. monocytogenes infection of BMM alone failed to induce considerable expression of monocyte chemoattractant protein (MCP)-1 at the mRNA or protein level, but co-treatment with IFN-gamma was necessary. Release of infection-triggered MIP-2, MIP-1alpha and KC was negatively regulated by IFN-gamma. Similarly, IL-4 stimulated MCP-1 release by infected macrophages but reduced production of MIP-1alpha, MIP-2 and KC. IL-10 turned out to be a general deactivator in terms of macrophage chemokine production. IL-13 had no effect on MIP-1alpha, MIP-2 and KC production by infected BMM, but slightly reduced MCP-1 release. By using IFN-gamma and IL-4 gene deletion mutant mice, in vivo regulation of these chemokines by IL-4 and IFN-gamma in listeriosis was studied. In summary, our results show that chemokines are produced by macrophages infected with L. monocytogenes, and that chemokine release is differentially regulated by the macrophage modulators IFN-gamma, IL-4, IL-10 and IL-13.